

BRIEF COMMUNICATION

Affected Members of Melanoma-Prone Families With Linkage to 9p21 but Lacking Mutations in *CDKN2A* Do Not Harbor Mutations in the Coding Regions of Either *CDKN2B* or *p19^{ARF}*

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Mutations in the gene encoding the cell cycle inhibitor *CDKN2A* have been identified in some melanoma kindreds linked to 9p21. However, many such families show no evidence of mutations in the coding regions of *CDKN2A*. In this study, we examined whether two other potential tumor suppressors, *CDKN2B* and *p19^{ARF}*, which also map within the 9p21 region, play a role in the development of familial melanoma. We found no mutations in the coding regions of either gene in melanoma-prone families with evidence of linkage to 9p21. We conclude either that another melanoma susceptibility gene exists within this chromosomal area or that mutations in noncoding regions of *CDKN2A*, *CDKN2B*, or *p19^{ARF}* predispose to melanoma. Genes Chromosom. Cancer 19:52-54, 1997. © 1997 Wiley-Liss, Inc.

The gene encoding the cell cycle inhibitor *CDKN2A* (also known as p16, MTS1, p16^{INK4A}, and CDK4I) has been mapped to human chromosome band 9p21, a region that contains one or more melanoma susceptibility genes (Kamb et al., 1994a). Germline mutations within the coding region of *CDKN2A* have been observed in affected members of families with inherited melanoma (Hussussian et al., 1994; Goldstein and Tucker, 1995; Gruis et al., 1995; Kamb, 1995; Liu et al., 1995; Walker et al., 1995). Moreover, the corresponding mutant proteins show diminished binding to CDK4 and, thus, block G1/S progression less effectively than does wild-type *CDKN2A* (Liu et al., 1995; Ranade et al., 1995; Reymond and Brent, 1995). However, the majority of familial melanoma kindreds do not have mutations in the coding region of *CDKN2A* (Kamb et al., 1994b; Ohta et al., 1994; Holland et al., 1995; Borg et al., 1996). Some of these families may bear mutations in genes at other loci. For example, two non-9p-linked families have been described with germline mutations in the *CDK4* gene (human chromosome 12q14; Zuo et al., 1996). In addition, some linkage analyses have identified candidate loci associated with inherited melanoma at 1p36 (Goldstein et al., 1993) and 6p (Walker et al., 1994). However, a considerable number of melanoma-prone families remain that lack mutations in the coding region of *CDKN2A* but that, nevertheless, show evidence of linkage to 9p21. These observa-

tions could be explained in either of two ways. First, a gene other than *CDKN2A* but within the 9p21 region could be involved in the genesis of melanoma. Alternatively, these families might have mutations in *CDKN2A* in areas outside the previously described coding regions.

The *CDKN2B* gene has been mapped to the 9p21 region approximately 25 kilobases centromeric to *CDKN2A* (Jen et al., 1994). Like the closely related *CDKN2A* protein, *CDKN2B* has also been shown to inhibit the catalytic activity of cyclin-D-CDK4 complexes in vitro and, thus, has the potential to function as a tumor suppressor (Hannon et al., 1994). In addition, a second potential tumor suppressor product, designated *p19^{ARF}* (alternate reading frame), is encoded by the *p16* gene (Duro et al., 1995; Mao et al., 1995; Stone et al., 1995b). The *p19^{ARF}* protein is derived from an alternatively spliced *p16* transcript (the β form), which contains the same exons 2 and 3 that are found in the mRNA encoding *CDKN2A*, but it employs a different exon 1 (referred to as E1β) that lies approximately 20 kilobases upstream of exon 2. Although the β transcript encodes a protein in a completely different reading frame relative to *p16^{INK4A}*, surprisingly,

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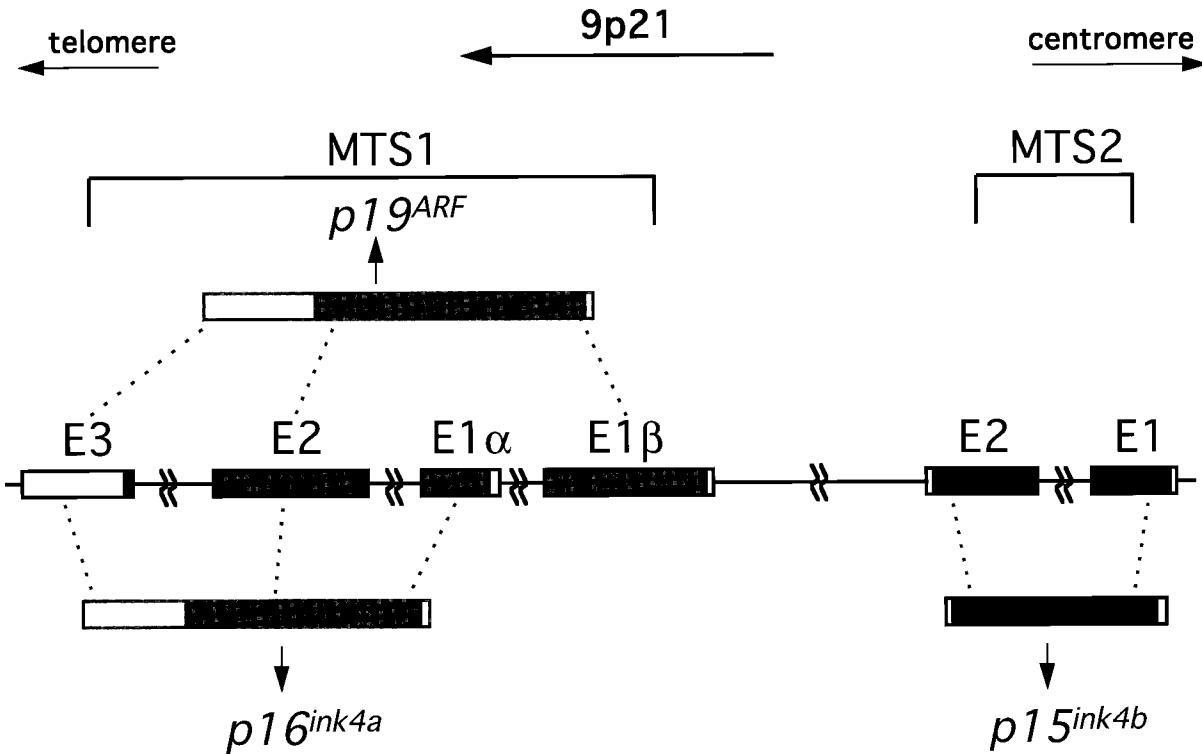


Figure 1. Genomic structure of proven (*CDKN2A*) and potential (*p19^{ARF}*, *CDKN2B*) tumor suppressor genes within the 9p21 chromosomal region.

the *p19^{ARF}* protein also blocks progression through the cell cycle. However, unlike the *CDKN2A* protein, the *p19^{ARF}* gene product does not associate with recombinant cyclin-D-CDK4 complexes; thus, these two proteins function independently as cell cycle regulators (Quelle et al., 1995). A schematic diagram of the *CDKN2A*, *p19^{ARF}*, and *CDKN2B* genomic structure is shown in Figure 1.

To determine whether mutations in either *CDKN2B* or *p19^{ARF}* predispose to the development of melanoma, we examined affected members of melanoma-prone families who were shown previously not to harbor functionally significant mutations in the coding regions of *CDKN2A*. A total of ten melanoma-prone families were evaluated. Selected probands from each family had been screened for mutations in exons 1 α , 2, and 3 of *CDKN2A* by SSCP and/or direct DNA sequencing (Hussussian et al., 1994). Although polymorphisms in the *CDKN2A* gene were detected in some of these families, none of these mutations has been shown to alter the function of the *CDKN2A* protein (Hussussian et al., 1994; Quelle et al., 1995; Ranade et al., 1995).

To facilitate discussion, we have placed these melanoma-prone families into two groups. The first group, comprised of six families (see Table 1), has been examined previously for linkage to the 9p21

TABLE 1. Maximum Lod Scores (Z_{\max}) Between *IFNA* and Either Melanoma or Melanoma/Dysplastic Nevus Syndrome and Conditional Probability (P) of Being Linked to *IFNA*¹

Family ID	CMM alone			CMM/DN		
	Z_{\max}	θ	P	Z_{\max}	θ	P
0377	1.6	0.0	0.99	2.1	0.0	0.99
2482	1.6	0.0	0.99	1.8	0.0	0.98
3311	0.2	0.0	0.75	1.4	0.0	0.93
0373	0.02	0.4	0.5	1.8	0.0	0.98
0909	0.5	0.0	0.85	0.3	0.0	0.63
2209	0.2	0.0	0.76	0.1	0.2	0.39

¹CMM/DN, cutaneous malignant melanoma/dysplastic nevus.

chromosomal region by the use of a highly polymorphic marker in the *IFNA* locus. Two of these families showed very strong evidence of linkage to 9p21 ($Z_{\max} = 1.6$; $P = 0.99$). Three families had Lod scores consistent with linkage to 9p21 (Z_{\max} ranging from 0.2 to 0.5; P values from 0.75 to 0.85). The remaining family (0373) showed small negative Lod scores when evaluated solely for cutaneous melanoma, but, when analyzed for the combined trait of cutaneous melanoma/dysplastic nevi, the family showed very strong evidence of linkage to this chromosomal region ($Z_{\max} = 1.8$; $P = 0.98$).

Because the centromeric end of the *IFNA* locus is approximately 500 Kb from the *CDKN2A* gene, we also determined whether affected individuals from the 9p-linked kindreds shared haplotypes across *CDKN2*. Families 0377 (markers IFNA, D9S171, and D9S126), 2482 [IFNA, *CDKN2* (436 mutation), and D9S126], and 0373 (IFNA, *CDKN2*, and D9S126) showed the same haplotypes across *CDKN2*. In the remaining three families, haplotype sharing could not be confirmed due to a lack of informative markers.

The second group of patients was comprised of four melanoma-prone families, each containing multiple affected members. These families have yet to be linked with any chromosomal region. Linkage studies on one of these families were uninformative, whereas the remaining three families have yet to be analyzed.

Genomic DNA from affected members of these families was screened by SSCP analysis. Sequences corresponding to exons 1 and 2 of *CDKN2B* were amplified with the same primers and under the same conditions as described previously (Liu et al., 1995). Exon 1 β sequences were generated as outlined by Stone et al. (1995b). We found no mutations in either *CDKN2B* or exon 1 β in any of these affected family members.

We conclude that germline mutations in exon 1 β and in the coding regions of *CDKN2B* are not responsible for melanoma susceptibility in 9p21-linked families. In agreement with these findings, Stone et al. (1995a,b) found no evidence for a mutation in either exon 1 β or *CDKN2B* in six additional families (distinct from those reported here) that showed significant haplotype sharing at 9p21 but lacked mutations in the *CDKN2A* coding sequence. The observations reported here, taken together with those of Stone et al., suggest two possible scenarios: Either mutations in noncoding regions of *CDKN2B*, *CDKN2A*, and/or *p19^{ARF}* play a role in the development of this disease, or another, as yet unidentified melanoma susceptibility gene exists within the 9p21 region.

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